

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference N.81753A SMW	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)	
International application No. PCT/EP 03/06552	International filing date (day/month/year) 20.06.2003	Priority date (day/month/year) 19.06.2002
International Patent Classification (IPC) or both national classification and IPC C12P7/64		
Applicant DSM IP ASSETS B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

I ☒ Basis of the opinion

II ☐ Priority

III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

IV ☐ Lack of unity of invention

V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

VI ☐ Certain documents cited

VII ☐ Certain defects in the international application

VIII ☐ Certain observations on the international application

Date of submission of the demand 19.01.2004	Date of completion of this report 05.08.2004
Name and mailing address of the international preliminary examining authority: <div style="display: flex; align-items: center;"> <div> European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465 </div> </div>	Authorized Officer Döpfer, K-P Telephone No. +49 89 2399-8547 <div style="text-align: right;"> </div>

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. **PCT/EP 03/06552**

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, Pages

1-19 as originally filed

Claims, Numbers

1-11 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

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**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability;
citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes: Claims	1-6
	No: Claims	7-11
Inventive step (IS)	Yes: Claims	1-6
	No: Claims	7-11
Industrial applicability (IA)	Yes: Claims	1-11
	No: Claims	

2. Citations and explanations

see separate sheet

**INTERNATIONAL PRELIMINARY
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International application No. PCT/EP 03/06552

Re Item I

Basis of the report

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

- D1: EP-A-1 035 211 (SUNTORY LTD) 13 September 2000 (2000-09-13) cited in the application
- D2: WO 96 21037 A (MARTEK BIOSCIENCES CORP ;KYLE DAVID J (US)) 11 July 1996 (1996-07-11) cited in the application
- D3: JANG HUNG-DER ET AL: 'Polyunsaturated fatty acid production with *Mortierella alpina* by solid substrate fermentation' BOTANICAL BULLETIN OF ACADEMIA SINICA (TAIPEI), vol. 41, no. 1, January 2000 (2000-01), pages 41-48, XP002270666 ISSN: 0006-8063
- D4: WO 92 13086 A (MARTEK CORP) 6 August 1992 (1992-08-06)
- D5: LINDBERG A ET AL: 'EFFECT OF TEMPERATURE AND GLUCOSE SUPPLY ON THE PRODUCTION OF POLYUNSATURATED FATTY ACIDS BY THE FUNGUS *MORTIERELLA ALPINA* CBC 343.66 IN FERMENTOR CULTURES' APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, SPRINGER VERLAG, BERLIN, DE, vol. 39, 1993, pages 450-455, XP002924350 ISSN: 0175-7598
- D6: YAMADA H ET AL: 'PRODUCTION OF DIHOMO-GAMMA-LINOLENIC ACID, ARACHIDONIC ACID AND EICOSAPENTAENOIC ACID BY FILAMENTOUS FUNGI', INDUSTRIAL APPLICATIONS OF SINGLE CELL OILS, XX, XX, PAGE(S) 118-138 XP001000789
- D7: WO 97 37032 A (WOLF JOHANNES HENDRIK ;SCHAAP ALBERT (NL); BIJL HENDRIK LOUIS (NL)) 9 October 1997 (1997-10-09)

2. Novelty and Inventive Step (Article 33(2)(3) PCT)

- 2.1 The present application addresses a microbiological process for the production of a polyunsaturated fatty acid (PUFA) characterised by a consumption rate of the growth rate-limiting carbon source which is greater than its rate of addition. Furthermore, the microorganism, preferably *Mortierella alpina*, metabolises fat(s) and/or lipids. After having reached a zero level of the carbon source the fermentation is allowed to continue. The PUFA is present in a microbial oil which is also claimed (with at least 50% content of arachidonic acid (ARA), having a

triglyceride content of at least 90%, a peroxide value of < 2.5 , an anisidine value of ≤ 1.0 , being hexane extracted and has a phospholipid content $< 5\%$).

Compositions containing said oil are claimed too.

- 2.2 None of the prior art documents D1 to D7 discloses a process comprising the steps disclosed in present claims 1 to 6. All documents using the microorganism *Mortierella alpina* do not use the modulation of carbon source in order to increase the yield of PUFA. Accordingly, present claims 1-6 are novel.
- 2.3 The subject-matter of present claims 7-11 concerning the microbial oil containing ARA and compositions comprising the same is not novel in view of prior art documents D1 (see claims 11, 12, 17-20) and D7 (see claims and example 22) which disclose a microbial oil with the characteristics mentioned in paragraph V.2.1 of this Written Opinion. Furthermore, the uses of present claims 10 and 11 are mentioned as well.
- 2.4 D5 is considered as representing the closest prior art. The content of this document is related to the effect of temperature and glucose supply on the production of PUFAs by the fungus *Mortierella alpina* CBS 343.66 in fermenter cultures. Also the influence of glucose exhaustion has been studied (see page 452, right-hand column, last paragraph to page 453, left-hand column, last paragraph; Fig 2 a,d; Table 5). D5 teaches that the content of ARA in the lipid fraction can be increased by glucose exhaustion, but the absolute yield of lipids is decreased.
- The problem underlying the present application can be seen as to provide an alternative method for the production of PUFAs, particularly ARA, which gives higher yields at reduced input.
- Neither D5 as closest prior art alone or in combination with any of the other prior art documents cited gives a qualified hint to the solution of the posed problem presented by the present application. The present method results in a microbial oil with remarkably high content of PUFAs, in particular ARA which could not be derived or expected from the prior art teachings. The subject-matter of present claims 1-6 is thus considered involving an inventive step.

3. Industrial applicability (Article 33(4) PCT)

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The subject-matter of present claims 1-11 appear to comply with the requirements of industrial applicability as stipulated in Article 33(4) PCT.